

WHAT IS CLAIMED IS:

Sub B² 1. A method of producing embryonic or stem-like cells comprising the following steps:

(i) inserting a donor differentiated human or mammalian cell or cell nucleus into a recipient animal oocyte, wherein such oocyte is derived from a different animal species than the human or mammalian cell under conditions suitable for the formation of a nuclear transfer (NT) unit, wherein the endogenous oocyte nucleus is removed or inactivated before, concurrent, or after introduction of donor cell or nucleus;

(ii) activating the resultant nuclear transfer unit;

(iii) additionally inserting into said oocyte cytoplasm derived from an oocyte or blastomere of the same species as the donor cell or nucleus ("compatible cytoplasm");

(iv) culturing said activated nuclear transfer unit until greater than the 2-cell developmental stage; and

(v) culturing cells obtained from said cultured NT units to obtain embryonic or stem-like cells.

2. The method of Claim 1, which further includes introducing mitochondrial DNA of the same species as the donor cell or nucleus into the recipient oocyte ("compatible mitochondria").

3. The method of Claim 1, wherein said cytoplasm is introduced before, concurrent, or after introduction of donor cell or nucleus.

4. The method of Claim 4, wherein said introduction occurs within about six hours of introduction of the donor cell or nucleus.

5. The method of Claim 4, wherein said oocyte is an immature oocyte

6. The method of Claim 5, wherein said oocyte is an immature human oocyte.

7. The method of Claim 5, wherein said immature human oocyte is matured *in vitro* prior to isolation of cytoplasm therefrom.

8. The method of Claim 4, wherein said immature oocyte is activated *in vitro* prior to isolation of cytoplasm therefrom.

9. The method of Claim 8, wherein said *in vitro* activation comprises contacting said oocyte with a compound that increases calcium levels.

10. The method of Claim 2, wherein all or part of the cytoplasm of the recipient oocyte is removed prior to introduction of cytoplasm from said at least one oocyte or blastomere of the same species as the donor cell or nucleus.

11. The method of Claim 1, wherein the cell inserted into the enucleated animal oocyte is a human cell.

12. The method of Claim 11, wherein said human cell is an adult cell.

13. The method of Claim 11, wherein said human cell is an epithelial cell, keratinocyte, lymphocyte or fibroblast.

14. The method of Claim 11, wherein the oocytes are obtained from a mammal.

15. The method of Claim 14, wherein the animal oocyte is obtained from an ungulate.

16. The method of Claim 15, wherein said ungulate is selected from the group consisting of bovine, ovine, porcine, equine, caprine, and buffalo.

17. The method of Claim 1, wherein the enucleated oocyte is matured prior to enucleation.

18. The method of Claim 1, wherein the fused nuclear transfer units are activated *in vitro*.

19. The method of Claim 1, wherein the activated nuclear transfer units are cultured on a feeder layer culture.

20. The method of Claim 19, wherein the feeder layer comprises fibroblasts.

21. The method of Claim 1, wherein in step (v) cells from a NT unit having 16 cells or more are cultured on a feeder cell layer.

22. The method of Claim 21, wherein said feeder cell layer comprises fibroblasts.

23. The method of Claim 22, wherein said fibroblasts comprise mouse embryonic fibroblasts.

24. The method of Claim 1, wherein the resultant embryonic or stem-like cells are induced to differentiate.

25. The method of Claim 11, wherein the resultant embryonic or stem-like cells are induced to differentiate.

26. The method of Claim 1, wherein fusion is effected by electrofusion.

5, 6 B³ 27. Embryonic or stem-like cells obtained according to the method of Claim 1.

28. Human embryonic or stem-like cells obtained according to the method of Claim 11.

29. Human embryonic or stem-like cells obtained according to the method of Claim 12.

30. Human embryonic or stem-like cells obtained according to the method of Claim 13.

31. Human embryonic or stem-like cells obtained according to the method of Claim 14.

32. ~~Human embryonic or stem-like cells obtained according to the method of Claim 15.~~

33. Differentiated human cells obtained by the method of Claim 25.

34. The differentiated human cells of Claim 33, which are selected from the group consisting of neural cells, hematopoietic cells, pancreatic cells, muscle cells, cartilage cells, urinary cells, liver cells, spleen cells, reproductive cells, skin cells, intestinal cells, and stomach cells.

35. ~~The differentiated human cells of Claim 33, which contain and express an inserted gene.~~

Sub 84 36. ~~The method of Claim 1, wherein a desired gene is inserted, removed or modified in said embryonic or stem-like cells.~~

37. The method of Claim 36, wherein the desired gene encodes a therapeutic enzyme, a growth factor or a cytokine.

38. The method of Claim 37, wherein said embryonic or stem-like cells are human embryonic or stem-like cells.

39. The method of Claim 36, wherein the desired gene is removed, modified or deleted by homologous recombination.

40. The method of Claim 1, wherein the donor cell is genetically modified to impair the development of at least one of endoderm, ectoderm and mesoderm.

41. The method of Claim 1, wherein the donor cell is genetically modified to increase differentiation efficiency.

42. The method of Claim 40, wherein the cultured nuclear transfer unit is cultured in a media containing at least one capsase inhibitor.

43. The method of Claim 1, wherein the donor cell expresses a detectable label that is indicative of the expression of a particular cyclin.

44. The method of Claim 40, wherein the donor cell has been modified to alter the expression of a gene selected from the group consisting of SRF, MESP-1, HNF-4, beta-1, integrin, MSD, GATA-6, GATA-4, RNA helicase A, and H beta 58.

45. The method of Claim 41, wherein said donor cell has been genetically modified to introduce a DNA that provides for expression of the Q7 and/or Q9 genes.

46. The method of Claim 45, wherein said gene or genes are operably linked to a regulatable promoter.

47. The method of Claim 1, wherein the donor cell has been genetically modified to inhibit apoptosis.

48. The method of Claim 47, wherein reduced apoptosis is provided by altering expression of one or more genes selected from the group consisting of Bad, Bok, BH3, Bik, Blk, Hrk, BNIP3, Gim_L, Bid, EGL-1, Bcl-XL, Bcl-w, Mcl-1, A1, Nr-13, BHRF-1, LMW5-HL, ORF16, Ks-Bcl-2, E1B-19K, and CED-9.

49. The method of Claim 48, wherein at least one of said genes is operably linked to an inducible promoter.

sub 8⁵ 50. ~~A mammalian somatic cell that expresses a DNA that encodes a detectable marker, the expression of which is linked to a particular cyclin.~~

51. The cell of Claim 50, wherein the cyclin is selected from the group consisting of cyclin D1, D2, D3, B1, B2, E, A and H.

52. The cell of Claim 50, wherein the detectable marker is a fluorescent polypeptide.

54. The cell of Claim 52, wherein said cell is a human, bovine or primate cell.

add B⁶ >